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Effect of high and low glycaemic index recovery diets on intramuscular lipid oxidation during aerobic exercise

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Intramyocellular lipid (IMCL) and plasma NEFA are important skeletal muscle fuel sources. By raising blood insulin concentrations, carbohydrate ingestion inhibits lipolysis and reduces circulating NEFA. We hypothesised that differences in the postprandial glycaemic and insulin response to carbohydrates (i.e. glycaemic index; GI) could alter NEFA availability and IMCL use during subsequent exercise. Endurance-trained individuals (n 7) cycled for 90 min at 70 % $\dot{V}O_{2\text{peak}}$ and then consumed either high GI (HGI) or low GI (LGI) meals over the following 12 h. The following day after an overnight fast, the 90 min cycle was repeated. IMCL content of the *vastus lateralis* was quantified using magnetic resonance spectroscopy before and after exercise. Blood samples were collected at 15 min intervals throughout exercise and analysed for NEFA, glycerol, glucose, insulin, and lactate. Substrate oxidation was calculated from expired air samples. The 90 min cycle resulted in >2-fold greater reduction in IMCL in the HGI trial (3.5 (SEM 1.0) mm/kg wet weight) than the LGI trial (1.6 (SEM 0.3) mm/kg wet weight, $P < 0.05$). During exercise, NEFA availability was reduced in the HGI trial compared to the LGI trial (area under curve 2.36 (SEM 0.14) mEq/l per h v. 3.14 (SEM 0.28) mEq/l per h, $P < 0.05$ respectively). No other differences were significant. The findings suggest that HGI carbohydrates reduce NEFA availability during exercise and increase reliance on IMCL as a substrate source during moderate intensity exercise.

Glycaemic index: Intramyocellular lipid: Magnetic resonance spectroscopy: Exercise: Skeletal muscle

During moderate intensity exercise, the energy for ATP homeostasis in skeletal muscle is primarily obtained from the oxidation of glycogen and lipid. Glycogen plays a pivotal role in producing energy and preventing the development of muscle fatigue in submaximal exercise^{1–4}. Intramuscular lipid levels are also readily oxidised during prolonged exercise^{5–7} and are elevated in endurance-trained individuals⁸. The rate of substrate oxidation, whether glycogen or lipid, is influenced by both existing pre-exercise substrate within the muscle⁹, and circulating substrate availability during exercise^{10–13}. Although considerable work has been undertaken on optimising glycogen storage with diet, less is known about the relative influence of diet upon both glycogen and lipid oxidation.

High levels of carbohydrate intake have been shown to improve glycogen repletion after exercise^{14,15}. An early study also reported that consuming carbohydrates which are rapidly absorbed into the circulatory system, termed high glycaemic index (HGI) carbohydrates, optimised the storage of plasma glucose as skeletal muscle glycogen following glycogen-depleting exercise¹⁶. It has also been reported that the postprandial hyperinsulinaemia accompanying high

carbohydrate intake reduces the rate of fat oxidation^{17–19}, increasing reliance upon glycogen oxidation during exercise. Although high postprandial levels of insulin may increase glycogen storage, the suppression of peripheral lipolysis may increase dependency upon intramuscular stores of glycogen and lipid during exercise²⁰.

Plasma NEFA are esterified to form intramyocellular lipid (IMCL) in non-contracting muscle and are oxidised during exercise^{21,22}. During moderate intensity exercise (40–65 % $\dot{V}O_{2\text{max}}$) fat oxidation contributes 40–60 % of the total energy expenditure^{5,6,17,23}. Of the total fat oxidised, 50–70 % is derived from plasma NEFA with the remaining proportion obtained from intramuscular and lipoprotein-derived TAG²⁴. The postprandial rise in insulin suppresses lipolysis, thereby decreasing circulating NEFA available for storage as IMCL and oxidation in skeletal muscle^{17,25}. Artificially elevating circulating NEFA levels increases lipid oxidation and reduces glycogen use in exercise^{10–13}, reinforcing the relationship between lipid and glycogen oxidation.

It is possible that optimising glycogen resynthesis with a HGI recovery diet occurs at the cost of compromised NEFA availability^{17–19}. Maintaining lipid availability with a low

Abbreviations: FFA, free fatty acids; GI, glycaemic index; HGI, high GI; LGI, low GI; IMCL, intramyocellular lipid; MRS, magnetic resonance spectroscopy; $\dot{V}O_{2\text{peak}}$, peak $\dot{V}O_2$.

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glycaemic index (LGI) diet may therefore assist endurance exercise performance. We hypothesised that LGI meals consumed after prolonged endurance exercise improve fatty acid availability and reduce reliance on IMCL in subsequent bouts of exercise. To test this hypothesis, LGI or HGI meals were consumed during a 24 h recovery period following 90 min of moderate intensity cycling. After an overnight fast, IMCL was examined using ^1H -magnetic resonance spectroscopy (MRS) before and after a second 90 min cycle. Venous blood samples were collected to observe changes in circulating lipids, glucose, insulin and lactate. Expired air samples were collected to evaluate changes in substrate use during exercise.

Subjects and methods

Subject information and initial testing

Seven endurance-trained male cyclists (30 (SEM 6) years of age, 80 (SEM 8) kg body weight) gave written informed consent. The study was approved by the Central Sydney Area Health, Human Ethics Research Committee, Sydney, Australia. Peak $\dot{V}\text{O}_2$ ($\dot{V}\text{O}_{2\text{peak}}$) was determined whilst cycling on a stationary cycle ergometer using four 5 min steady state stages (100, 150, 200 and 250 W) followed by a progressive increase in work of 10 W/min until voluntary exhaustion and averaged 4.6 (SEM 0.2) l/min. All exercise tests were conducted on the same electronically braked cycle ergometer (Tunturi E85, Tunturi, Turku, Finland).

Pre-trial exercise

After a 12 h fast, participants undertook a 90 min cycle at 70 % $\dot{V}\text{O}_{2\text{peak}}$ in the laboratory starting at 08.00 hours. Work rate (W) at 70 % $\dot{V}\text{O}_{2\text{peak}}$ was calculated for each individual from the linear function of O_2 uptake at the four steady-state work rates and maximal O_2 uptake against the work rate (W) of the four steady-state work rates and maximal power output. Mean work rate at 70 % $\dot{V}\text{O}_{2\text{peak}}$ was 216 (SEM 8) W. During the cycle, water was provided *ad libitum*. During subsequent cycle trials, the same amount of water was consumed.

Dietary manipulation

At the end of the first 90 min cycle, participants were provided with food for the following 24 h containing either a HGI or LGI carbohydrate component. The treatment order of the interventions was randomised. Carbohydrate was provided at 8 g/kg body mass with protein and fat content constituting 11 and 17 % of energy respectively (see Table 1 for details). The glycaemic index (GI) values were calculated from international tables²⁶. Breakfast was provided at the research facility. Packaged lunch, dinner and snacks were provided for the subject to consume at home. Water could be consumed *ad libitum* with no other drinks allowed. Participants were asked to only eat the food provided and to finish eating the food before 21.00 hours that night.

Experimental trial protocol

Participants arrived in the laboratory 24 h after the first 90 min cycle participants arrived in the laboratory and were weighed in minimal clothing. An indwelling cannula was then inserted into the cephalic vein for blood sampling. Magnetic resonance examinations were completed 0.5 h before and 1 h after the end of the experimental trial cycle (see later). Following a 10 min warm-up at a self-selected pace, participants cycled for 90 min at 70 % of their $\dot{V}\text{O}_{2\text{peak}}$. Expired air samples were collected for 2 min at 15, 30, 45, 60, 75 and 90 min time points and analysed online for expired fractions of O_2 , CO_2 and ventilation (Cortex Biophysik, Leipzig, Germany). Substrate oxidation rates were calculated using non-protein

Table 1. Nutrient content of the high (H) and low (L) glycaemic index (GI) diets (for a 70 kg person)

Meal component*	Diet	
	HGI	LGI
Breakfast		
Corn flakes (g)	60	–
White bread (g)	70	–
Jam (g)	30	–
Margarine (g)	5	–
Carbonated glucose drink (ml)	140	–
Skimmed milk (ml)	250	250
Guardian Cereal (g)	–	85
Apple (g)	–	70
Canned peaches (g)	–	130
Yoghurt (g)	–	100
Apple juice (ml)	–	400
Lunch / Dinner		
Turkey (g)	100	100
Lettuce (g)	50	50
Cheese (g)	50	50
White bread (g)	154	–
Banana (g)	175	–
Carbonated glucose drink (mL)	200	–
Wholemeal pasta (g)	–	150
Pasta sauce (g)	–	170
Pear (g)	–	100
Apple juice (g)	–	145
Snacks		
Carbonated glucose drink (ml)	95	–
Grain snack bar (g)	77	–
Apple juice (ml)	–	550
Fruit snack (g)	–	80
Macronutrient content		
Energy (KJ)	3244	3263
Carbohydrate (g)	561	555
Fat (g)	54	53
Protein (g)	115	133
GI	73	35

* Cornflakes (Kellogg's, Melbourne, Australia); White bread (Sunblest, Tip Top bakeries, Sydney, Australia); Jam (Cottees strawberry conserve, Cottees, Melbourne, Australia); Margarine (Devondale extra soft spread, Murray Goulburn, Melbourne, Australia); Carbonated glucose drink (Lucozade, GlaxoSmithKline, Boronia, Australia); Guardian Cereal (Kellogg's, Melbourne, Australia); Canned peaches (Goulburn Valley peach slices in natural juice (no added sugar); Yoghurt (Yoplait no fat fruit yoghurt, strawberry flavour if possible; National Foods, Sydney, Australia); Apple juice (Coles Farmland Apple Juice, no added sugar; Coles, Melbourne, Australia); Chicken (Castlemain shaved chicken breast, 97 % fat free; KR Castlemaine, Castlemaine, Australia); Cheese (Coles Farmland tasty cheddar cheese; Coles, Melbourne, Australia); Wholemeal pasta (San Remo wholemeal pasta spirals; San Remo, Sydney, Australia); Tomato sauce (Dolmio, Sydney, Australia); Grain snack bar (K-Time mixed berry bars; Kellogg's, Melbourne, Australia); Fruit snack (Arnott's Snack right fruit slice, sultana; Arnott's, Sydney, Australia).

stoichiometric equations²⁷. At rest and during each of the expired gas collections, 5 ml blood was collected into EDTA tubes and immediately centrifuged for 10 min at 4°C. Plasma was then extracted and stored at -80°C for later analysis.

Intramyocellular lipid assessment

With the subject supine, a vitamin-E capsule was placed equidistant between the caudal tip of the patella and the caudal head of the femur over the *vastus lateralis* and marked with indelible ink. Image-guided (from the vitamin-E capsule), localised proton magnetic resonance spectra of the right *vastus lateralis* muscle were generated on a 1.5 Tesla Philips magnetic resonance scanner (Philips Medical Systems, Best, The Netherlands). The subject lay on his right-hand side with a 10 cm C3 flex coil (Philips Medical Systems, Best, The Netherlands) positioned over the vitamin-E capsule. The magnetic field was optimised using a fully automated shim routine to ensure magnetic field homogeneity. Water suppressed and unsuppressed spectra were collected from a $1.5 \times 1.5 \times 2.0 \text{ cm}^2$ voxel using point resolved echo sequence spectroscopy (PRESS; echo time 32 ms, relaxation time 5 s).

Quantification of spectra

Calculation of metabolite (from water-suppressed spectra) and water (from water-unsuppressed spectra) resonances was performed using the java-based magnetic resonance user interface (jMRUI version 2.0)^{28,29}. Following manual first and second order phase correction, spectra were analysed using a non-linear least squares algorithm (AMARES)³⁰. With creatine CH-3 resonance selected as a reference at 3.02 ppm, eight other resonances were calculated: IMCL-CH₃ (0.88 ppm), extra-myocellular lipid (EMCL)-CH₃ (1.19 ppm), IMCL-CH₂ (1.28 ppm), EMCL-CH₂ (1.53), L-acetate 2.08, fatty acid-CH₂ (2.24 ppm), trimethylacetate group (3.19 ppm), and taurine (3.37 ppm). From the non-water suppressed spectra, only the water peak was quantified. The signal amplitude IMCL-CH₂ was corrected for T1^{31,32} and T2³³ relaxation times reported in the literature under equivalent conditions. For absolute quantification of IMCL, metabolites were then corrected for proton content and the IMCL-CH₂: water ratio (from the unsuppressed water spectra). Values were then converted into mm/kg wet weight, assuming a water concentration of 55 mm/kg wet weight and a tissue water fraction of 0.81, as previously described³⁴ (CV 0.71 mm/kg wet weight).

Blood assays

Plasma samples were analysed for glucose using a hexokinase enzymatic assay (Roche Diagnostic Systems, Sydney, Australia); insulin by a solid-phase, antibody-coated tube radioimmunoassay (Coat-A-Count Insulin RIA kit, Diagnostic Products Corporation, Los Angeles, CA, USA); lactate by enzymatic test (Roche Diagnostic Systems); glycerol using a colorimetric assay (RANDOX Laboratories Ltd, Crumlin, UK); free fatty acids (FFA) by a commercially available enzymatic colorimetric test kit (NEFA-HA; Wako Pure Chemical Industries Ltd, Osaka, Japan).

Statistical analysis

All statistical calculations were performed using SPSS version 11 (SPSS Inc., Chicago, IL, USA). A two-way analysis of variance (time and treatment) was used to assess metabolic and physiological differences between groups. A *post-hoc* Bonferroni step-wise correction was performed at the location of the variance. Statistical significance was accepted at $P < 0.05$. Data are presented as means with their standard error unless otherwise stated.

Results

Substrate oxidation

Whole-body fat oxidation increased and carbohydrate oxidation decreased over the 90 min of exercise (Fig. 1). During the HGI trial 197 (SEM 23) g carbohydrate and 63 (SEM 5) g fat were oxidised. During the LGI trial, 169 (SEM 11) g carbohydrate and 76 (SEM 9) g fat were oxidised. Lipid oxidation represented 37 (SEM 6) % of the total substrate oxidation in the HGI trial and 47 (SEM 8) % in the LGI trial. Despite trends in the predicted direction, none of the differences in substrate oxidation reached statistical significance (area under curve, $P = 0.13$).

Blood parameters

After an early decline, plasma FFA showed a progressive rise throughout exercise in both HGI (0.32 (SEM 0.06) at baseline to 0.67 (SEM 0.05) mmol/l, $P < 0.01$) and LGI groups (0.57 (SEM 0.14) at baseline to 0.81 (SEM 0.11) mmol/l, $P < 0.01$) (Fig. 2 (A)). For the first 60 min of exercise, levels of circulatory FFA were higher in the LGI trial than in the HGI trial ($P < 0.05$; Fig. 2 (A)). Plasma glycerol concentrations showed a progressive rise throughout exercise in the LGI (84 (SEM 17) at baseline to 483 (SEM 48) $\mu\text{mol/l}$, $P < 0.01$) and HGI trials (88 (SEM 32) at baseline to 341 $\mu\text{mol/l}$, $P < 0.05$), with higher concentrations shown in the LGI group at 15 and 90 min ($P < 0.05$; Fig. 2 (B)).

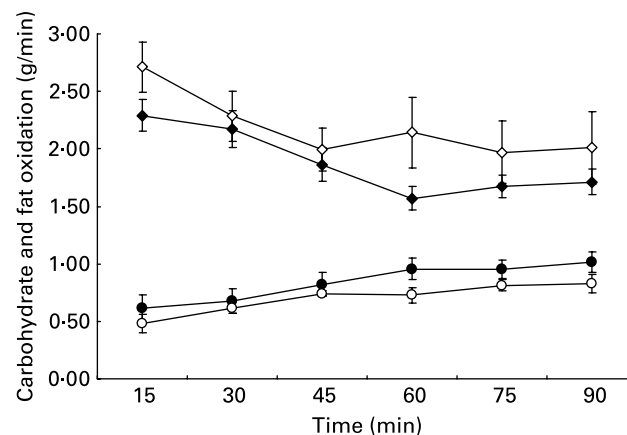


Fig. 1. Calculated carbohydrate (\diamond , \blacklozenge) and fat oxidation (\circ , \bullet) every 15 min whilst cycling at 70% $\dot{V}O_{2\text{peak}}$ following a high glycaemic index (\diamond , \circ) or low glycaemic index (\blacklozenge , \bullet) diet. Values are means with their standard errors shown by vertical bars.

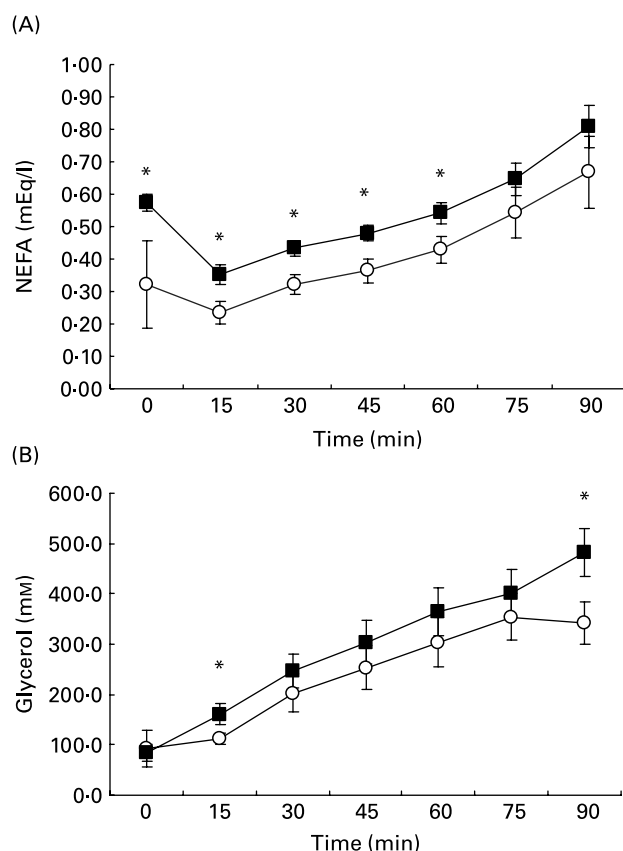


Fig. 2. Plasma NEFA (A) and glycerol (B) at rest and every 15 min whilst cycling at 70% $\dot{V}O_{2peak}$ following a high glycaemic index (HGI; ○) or low glycaemic index (LGI; ■) diet. Values are means with their standard errors shown by vertical bars. Mean values were significantly different from HGI relevant time point: * $P < 0.05$.

After an initial 45 min stability, there was a progressive decline in circulating glucose ($P < 0.01$; Fig. 3 (A)). Plasma glucose concentrations were higher in the LGI trial at 75 and 90 min ($P < 0.05$; Fig. 2 (A)). Insulin concentrations decreased progressively throughout exercise in both trials ($P < 0.01$; Fig. 3 (B)). After an initial rise from baseline, lactate concentrations remained stable throughout both trials (Fig. 3 (C)). Although mean lactate concentrations were consistently higher in the HGI trial, this did not achieve significance ($P = 0.07$). Plasma cortisol levels increased through exercise although there were no differences between the HGI and LGI trial ($P > 0.05$; Fig. 3 (D)).

Intramyocellular lipid

Exercise produced a significant reduction in IMCL in both the HGI (from 7.3 (SEM 1.4) at baseline to 3.8 (SEM 0.9) mm/kg wet weight, $P < 0.05$) and LGI (from 5.9 (SEM 0.8) at baseline to 4.4 (SEM 0.8) mm/kg wet weight, $P < 0.05$) groups over the 90 min of exercise (Fig. 4). The HGI group therefore showed a greater dependence on IMCL (3.5 (SEM 1.0) mm/kg wet weight) than the LGI group (1.6 (SEM 0.3) mm/kg wet weight; Fig. 4). There was no significant difference between baseline IMCL ($P = 0.08$) or end exercise IMCL ($P = 0.06$) between the LGI and HGI groups.

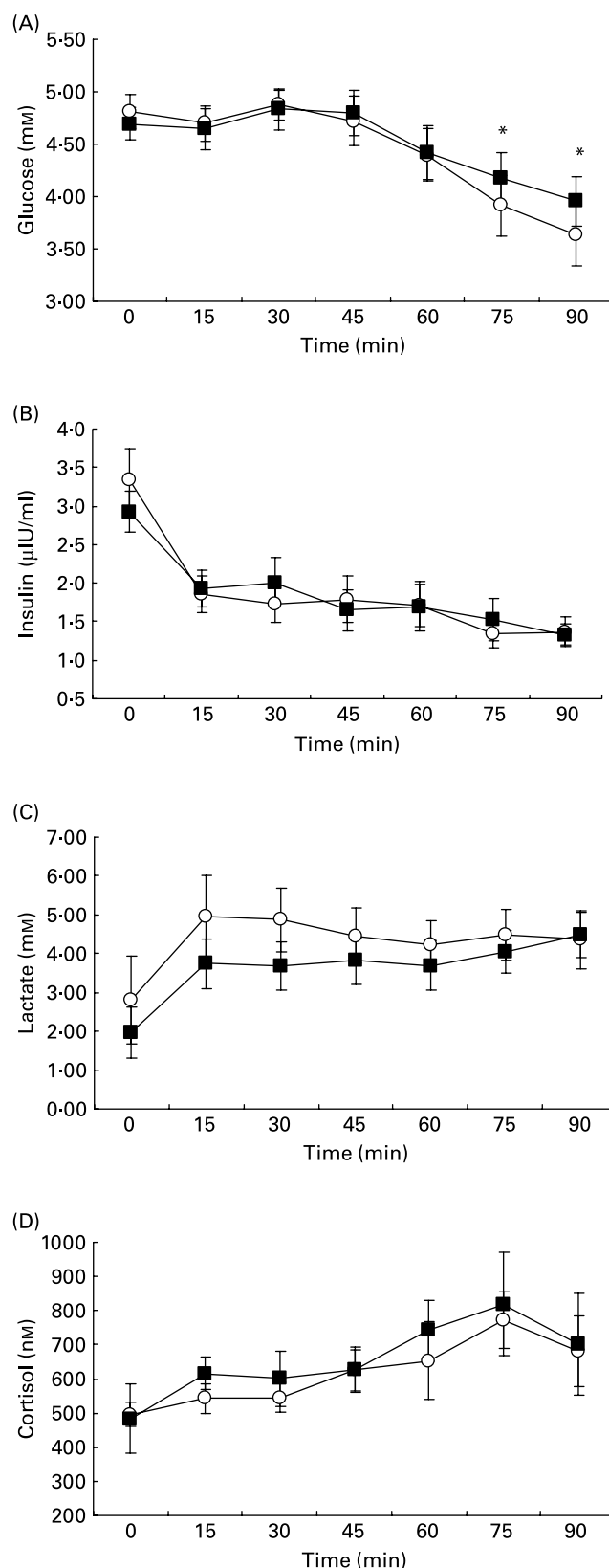


Fig. 3. Plasma glucose (A), insulin (B), lactate (C) and cortisol (D) at rest and every 15 min whilst cycling at 70% $\dot{V}O_{2peak}$ following a high glycaemic index (HGI; ○) or low glycaemic index (LGI; ■) diet. Values are means with their standard errors shown by vertical bars. Mean values were significantly different from HGI relevant time point: * $P < 0.05$.

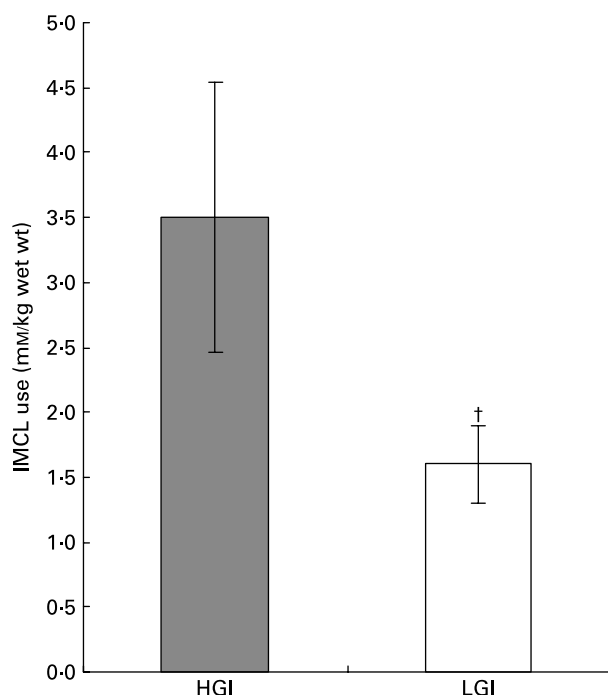


Fig. 4. Intramyocellular lipid (IMCL) use whilst cycling at 70% $\dot{V}O_{2peak}$ following a high (H) or low (L) glycaemic index (GI) diet. Values are means with their standard errors shown by vertical bars. Mean values were significantly different from HGI. $\dagger P < 0.05$.

Discussion

This study shows that the GI of the diet consumed in the recovery period influences substrate availability and utilisation in subsequent prolonged exercise. Circulating fatty acid availability during exercise was about 34% greater following a LGI diet compared with the HGI diet (quantified as area under the curve). The use of IMCL as a substrate source was >2-fold higher after the HGI meals than the LGI meals. Combined, these results suggest that consuming a LGI diet between bouts of prolonged strenuous exercise reduces dependence on intramuscular lipid stores and increases the use of circulating plasma FFA derived from other fat stores as a fuel source. In competitive sports and search-and-rescue operations, LGI meals might therefore improve final outcomes.

A novel aspect of the current study is the use of MRS to assess changes in IMCL following carbohydrate manipulation. The LGI diet was associated with a decreased IMCL oxidation and increased NEFA availability during exercise. In contracting skeletal muscle, the rate of NEFA uptake and oxidation is increased, preserving IMCL stores²². At rest, plasma NEFA are esterified in skeletal muscle to form IMCL^{22,35}. Indeed, dietary intakes high in fat (40–60% fat) promote IMCL storage^{36–38} while diets high in carbohydrate (5–25% fat) reduce IMCL storage^{38,39}.

Although the amount of IMCL oxidised during exercise was different between the treatment groups, the HGI group had a higher mean IMCL content before exercise and lower IMCL content after exercise, though these did not achieve statistical significance ($P=0.08$ and $P=0.06$ respectively).

It is possible that the higher starting levels of IMCL in the HGI group could aid subsequent IMCL oxidation⁹, theoretically, through an increase in the substrate–enzyme interface⁴⁰. The higher starting levels of IMCL in the HGI group would appear paradoxical, as a HGI diet should decrease NEFA availability to replenish IMCL in recovery from exercise¹⁹. However, the design of the present study cannot conclusively provide any explanation for these observations, as the study: (1) may be underpowered to detect changes in IMCL storage between groups, and (2) did not track postprandial lipid availability and oxidation over the recovery period. Irrespective of these comments, our data suggest that not only is the amount of carbohydrate and fat intake influential in determining substrate storage and use, but that the GI of the carbohydrate can change the availability and use of lipids during exercise. The observation of a trend towards differences in IMCL at the start and end of exercise between diets reinforces the need for further studies observing the influence of postprandial signalling and lipid availability upon IMCL storage.

Analysis of the indirect calorimetry data shows that there is a progressive increase in lipid oxidation and a decline in carbohydrate utilisation over the exercise period. The decline in carbohydrate utilisation has been attributed to a decrease in muscle glycogen utilisation and an increased uptake and use of plasma glucose⁶. Conversely, as plasma NEFA availability is reported to determine NEFA oxidation²¹, the progressive rise in plasma NEFA is likely to reflect an increase in NEFA oxidation. Studies using Intralipid infusion to elevate plasma NEFA concentrations show an increase in lipid oxidation and a reduced glycogen use in exercise^{10–13}, suggesting that FFA availability can influence substrate use in skeletal muscle. In further support, use of the antilipolytic agent nicotinic acid reduces plasma fatty acid and increases dependence on glycogen during exercise⁴¹. Despite this evidence, the pathways which control the relative contribution of carbohydrate and fat as substrate sources during metabolism are not completely understood⁴². However, the sustained suppression of circulating FFA during prolonged exercise may in part explain the increased reliance upon IMCL as a substrate source following a HGI diet.

Elevated insulin concentrations suppress lipolysis at rest and during subsequent exercise, reducing circulating levels of NEFA¹⁷. In line with this, acute intake of carbohydrate before exercise (<4 h) has been shown to decrease FFA availability and increase glycogen dependence during exercise²⁵. Altering the GI of carbohydrates consumed before exercise has been shown to influence substrate oxidation during exercise. Studies have reported that consuming a LGI breakfast 3 h before endurance exercise resulted in an increase in FFA availability and hence an increase in fat oxidation and decrease in carbohydrate oxidation compared to when a HGI breakfast is consumed^{18,43,44}. In the current study, an increase in FFA availability was observed during exercise the day after consuming a LGI diet. Interestingly, the elevated circulatory NEFA observed at rest and during exercise in the LGI group occurred independent of any differences in circulating insulin between the treatment groups. This is suggestive of heightened or dampened sensitivity of the adipose tissue to insulin following exposure to high (HGI) or moderate (LGI) exposure to insulin⁴⁵, respectively, the previous day.

Plasma glucose levels were stable over the first 45 min of exercise but then steadily declined. In the final 30 min, the decline was attenuated in the LGI group in comparison to the HGI group. It is likely that the difference represents increased dependence on glucose as the source of fuel in the HGI group⁴⁶. It is possible that both liver and muscle glycogen stores are being depleted faster but little is known about the relative roles of each. Liver glycogen stores appear to be important. Using ¹³C magnetic resonance spectroscopy⁴⁷, glycogen content of the liver following exhaustive exercise was found to predict subsequent exercise capacity. Further ¹³C-MRS studies are needed to determine the relative importance of different fuel sources.

Our observation of a significant decrease in IMCL in both LGI and HGI groups with 90 min of exercise demonstrates that IMCL is a major substrate source during prolonged exercise. This observation is in line with previous studies using isotope-labelled FFA^{5,9}, muscle biopsy^{6,9} and magnetic resonance^{6,7,9} analysis techniques. The use of IMCL as a substrate source during exercise has been the subject of much recent debate, with the inability of early studies to discriminate between intra- and extra-cellular lipids using biochemical analysis being central to this misunderstanding⁴⁸. The problem is clearly demonstrated by Howald *et al.*⁴⁹ who showed strong correlation between electron microscopic morphometry and MRS (r 0.93), demonstrating that both of these techniques are accurate in determining IMCL. However, the correlation between IMCL determined by biochemical analysis from muscle biopsy was weak with electron microscopic morphometry (r 0.41) and MRS (r 0.47). Our findings show that not only is IMCL (as assessed by ¹H-MRS) an important substrate during exercise, but also that the origin of lipid oxidised during exercise, whether intra- or extra-muscular, can be influenced by the composition of diet during the previous 24 h. Future studies combining the use of ¹H-MRS measurement of IMCL with ¹³C-MRS measurements of glycogen will add further to our understanding of this complex area.

In summary, the GI of carbohydrates consumed after prolonged exercise had a significant effect on substrate utilisation during subsequent exercise. The availability of plasma FFA was increased and IMCL use decreased when a LGI diet was consumed over a 24 h period compared to when a HGI diet was consumed. Further research is required to investigate whether the change in lipid oxidation with diets differing in GI alters glycogen usage and whether these changes in turn hold benefits for endurance-exercise performance.

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